Substance Group:	**Group 10 –Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide**

201-16491B

Summary Prepared by:	**Petroleum Additives Panel** **Health, Environmental and Regulatory Task Group**
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1.0 Physical-Chemical Properties

Robust Summary 10- Partition Coefficient -1

Test Substance	
CAS #	398141-87-2 (18760-44-6)
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline followed	OPPTS 830.7570, Partition Coefficient (n-Octanol/Water), Estimation by Liquid Chromatography, This method is based on procedures presented in the OECD Guideline for the Testing of Chemicals, 117: Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method; TSCA Title 40 of the Code of Federal Regulations, Part 796, Section 1570: Partition Coefficient (n-Octanol/Water) – Estimation by Liquid Chromatography and Council of the European Communities Directive 92/69/EEC Annex V, Method A.8
Test Type	Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method
GLP	Yes
Year (Study Performed)	2005
Log P_{ow} Reference Standards	Acetanilide: Purity: 99.91%, 50 µg/L, Guideline reported log P_{ow} = 1.0 Toluene, Purity: 99.94%, 100 µg/L, Guideline reported log P_{ow} = 2.7 Naphthalene: Purity: 99.9%, 24.6 µg/L, Guideline reported log P_{ow} = 3.6 Phenanthrene: Purity: 100%, 25 µg/L, Guideline reported log P_{ow} = 4.5 2-Nitrophenol: Purity: 99.3%, 50 µg/L, Guideline reported log P_{ow} = 1.8 DDT, Purity: 99.3%, 50 µg/L, Guideline reported log P_{ow} = 6.5
Dead Time Reference Standard	Thiourea, 99.5% 50µg/L, mean retention time 1.674 minutes
Calculations	Partition coefficient was determined from the capacity factor k′. The capacity factor (k′), was calculated for each of the test substance chromatographic components and each reference standard using the following equation: $k' = (tR - t0)/t0$ where tR was the retention time of the test substance or reference standard and t0 was the column dead time established with thiourea. A correlation graph of Log k′ versus Log Kow for the reference standards was plotted and fitted to a regression equation in the form y = mx + b. Log Kow for each component of the test substance was calculated by substituting the calculated logarithm of the capacity factor for each component into the linear regression equation for the calibration curve.
HPLC Conditions	Instrument: Agilent Series 1100 High Performance Liquid Chromatograph (HPLC) with an Agilent Series 1100 Variable Wavelength (UV) Detector Column: YMC-Pack ODS-AM C18 (150 mm x 4.6 mm, 3-µm particle size) Stop Time: 30.0 minutes Flow Rate: 1.0 mL/minute

	Oven Temperature: 40°C Mobile Phase: Acetonitrile:0.1% H3PO4; 75:25, v/v Injection Phase: 100 µL Primary Analytical Wavelength: 210 nm
Method Comments	The reference standards were used to generate a Log Kow calibration curve. Calibration stock solutions of the individual reference standards were prepared separately by dissolving each in acetonitrile. Calibration standard solutions were prepared from these stock solutions of the reference standards by diluting appropriately in 75% acetonitrile: 25% HPLC-grade water (v/v). The concentrations of the reference standards were adjusted, as necessary, to give a detector response of at least 900 milli-absorbance units for peak height. A stock solution of the test substance was prepared by weighing 0.1250 g of the test substance into a 20-mL glass scintillation vial. The test substance was then quantitatively transferred into a 25.0-mL volumetric flask. The stock solution was brought to volume using acetonitrile. This primary stock solution contained 5.00 mg/mL. Subsequent dilution of the acetonitrile stock solution yielded a 1000 µg /mL solution that was prepared in 75% acetonitrile: 25% HPLC-grade water. Each of the triplicate injections (100 µL) of this solution gave a detector response of at least 100 milli-absorbance units for the peak height of the dominant peak. Along with analysis of reference standards, a standard of thiourea was injected in duplicate (once near the beginning and once near the end of the HPLC sequence) to determine the analytical column dead time (t0) for use in calculating capacity factors (k′) of the reference standards and the test substance. The mean retention time of the duplicate thiourea standard injections was 1.674 minutes. Six additional reference standards were analyzed with the test substance. The reference standard series was injected at the beginning and at the end of the HPLC sequence. The capacity factors of each standard were calculated based upon their retention times. The test substance was sequentially injected in triplicate.

Results

The test substance eluted as eight resolved peaks. The peak retention times for the test substance ranged from 1.796 ± 0.000577 minutes to 7.266 ± 0.0306 minutes. The partition coefficients (Log Kow) for the test substance were calculated as (-0.91), 2.06, 2.81, 3.81, 4.38, 4.50, 4.62 and 5.48. The value in parentheses was extrapolated. Using the measured peak area for each calculated partition coefficient, the weighted mean Log Kow for the test substance was 1.19.

The mean retention times and Log_{10} P_{ow} values of the reference materials and test substance were as follows:

	Mean Retention Time (min)	Log_{10} P_{ow}
Acetanilide	1.932	1.0[a]
Toluene	3.296	2.7[a]
Naphthalene	3.640	3.6[a]
Phenanthrene	5.101	4.5[a]
2-Nitrophenol	2.314	1.8[a]
DDT	9.824	6.5[a]
Test Substance		
Peak 1	1.796	-0.91[b]
Peak 2	2.396	2.06
Peak 3	2.806	2.81
Peak 4	3.733	3.81
Peak 5	4.566	4.38
Peak 6	4.791	4.50
Peak 7	5.023	4.62
Peak 8	7.266	5.48
Overall Weighted Mean		1.19

[a]Literature values
[b]Extrapolated value

Conclusions	Under the chromatographic conditions specified in guidelines, the test substance eluted as eight resolved peaks. Using the measured peak area for each calculated partition coefficient, the weighted mean Log Kow for the test substance was determined to be 1.19.
Data Quality	Reliable without restriction.
References	Lezotte & Nixon. (2006.) "Determination of *n*-Octanol/Water Partition Coefficient." Wildlife International Project No.: 264C-104
Other	Updated: 7/25/2005

Robust Summary 10 -Water Solubility-2

<table>
<tr><td>CAS No.</td><td>18760-44-6</td></tr>
<tr><td>Test Substance Name</td><td>Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide</td></tr>
<tr><td>Method/Guideline</td><td>EEC Commission Directive 92/69/EEC Method A6 Water solubility</td></tr>
<tr><td>GLP (Y/N)</td><td>Yes</td></tr>
<tr><td>Year</td><td>2002</td></tr>
<tr><td>Remarks for Test Conditions</td><td>To each of three conical flasks, distilled water and test substance were added. The flasks were stirred at 30°C, one each for 24, 48 and 72 hours, and after standing at 20°C the contents of the flasks were centrifuged at 10000 rpm for 30 minutes. The concentration of the test material in the sample solutions was determined by gas chromatography. Duplicate 200 ml aliquots of each sample were extracted with three 30 mL portions of dichloromethane. Extracts were filtered through anhydrous sodium sulphate. The combined extracts were then evaporated to dryness and the residue re-dissolved in 5 mL of tetrahydrofuran. Duplicate standard solutions of test material were prepared in tetrahydrofuran at a nominal concentration of 1.00×10^{3} mg/L</td></tr>
<tr><td>Results</td><td>
<table>
<tr><th>Sample No.</th><th>Time Shaken at 30°C</th><th>Equilibration Time at 20°C</th><th>Concentration (g/l)</th><th>pH</th></tr>
<tr><td>1</td><td>24 hours</td><td>24 hours</td><td>5.50×10^{-2}</td><td>4.7</td></tr>
<tr><td>2</td><td>48 hours</td><td>24 hours</td><td>5.05×10^{-2}</td><td>5.3</td></tr>
<tr><td>3</td><td>72 hours</td><td>24 hours</td><td>5.66×10^{-2}</td><td>4.9</td></tr>
</table>

Mean concentration 5.4×10^{-2} g/l at 20°C $\pm$ 0.5°C

The analytical method was validated with respect to linearity and recovery of the test material from aqueous media. Instrument response was linear.</td></tr>
<tr><td>Conclusions</td><td>The water solubility of the test material was determined to be 5.4×10^{-2} g/l at 20°C $\pm$ 0.5°C</td></tr>
<tr><td>Data Quality</td><td>Reliable without restriction</td></tr>
<tr><td>References</td><td>Confidential business information</td></tr>
<tr><td>Other</td><td>November 21, 2002</td></tr>
</table>

2.0 Environmental Fate

2.1 Biodegradation

Robust Summary 10-BioDeg-1

Test Substance	
CAS #	18760-44-6
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline Followed	OECD 301B, Ready Biodegradability, Modified Sturm Test; ASTM Test Method D 5864-95.
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (study performed)	1997
Contact time (units)	28 days
Test apparatus	Six glass 4-liter Erlenmeyer
Inoculum	Activated sewage sludge from a domestic wastewater treatment plant and soil filtrate prepared per test guideline. Six adaptation cultures were prepared. The inoculum was combined with 900 mL of test medium within a 2-liter flask. Solutions were continuously aerated with CO_2 free air and the test substance was incrementally added at concentrations of 4, 8 and 8 mg C/L on days 0, 7 and 11. (This adaptation of the inoculum to the test material is not called for in the OECD Guideline. This deviation from the Guideline was not considered sufficient to invalidate the study.) On day 14 a composite culture was prepared and homogenized. A standard plate count was performed. Plates were incubated at $20^{O}C$ for 48 hours.
Replicates:	*All groups tested in triplicate*
Temperature of incubation:	20± 3°C
Dosing procedure:	Neat test chemical was gravimetrically added to glass cover slips, which were then added to culture medium in test vessels.
Study initiation:	Test flasks provided with 50-100 mL/minute CO_2 free air and mixed with a magnetic stirrer. The CO_2 produced from the degradation of organic carbon sources within each test chamber was trapped as K_2CO_3 in the KOH solution and measured using a carbon analyzer.
Sampling:	Days 4, 7, 12, 14, 19, 22 and 29 (after acidification on day 28)
Concentration of test substance:	10 mg carbon (C)/L weighed directly onto tared glass slides and placed into each test substance flask.
Controls:	Blank and positive controls used per guideline. Positive control was canola oil added to the control vessel at a loading of 10 mg C/L.
Analytical method:	The CO_2 produced from the degradation of organic carbon sources within each test chamber was trapped as K_2CO_3 in the KOH solution and measured using a carbon analyzer.
Study termination:	The pH of the content of each test flask was determined. The flasks were then acidified with 3 ml of concentrated hydrochloric acid to drive off inorganic carbonate. The chambers were aerated overnight and then the trapping solution closest to the test chamber was analyzed for inorganic carbon.

Method of calculating biodegradation values:	Percent biodegradation calculated as percent ratio of cumulative net carbon dioxide to theoretical carbon dioxide as determined from elemental analysis of test material.
Results	The test substance was not considered readily biodegradable under the criteria that requires 60% biodegradation within 28 days, achieved within 10 days of reaching 10% biodegradation. The CO_2 production from the reference chemical exceeded the 60% of theoretical necessary to consider the test valid.
Degradation % After Time	Test substance: 9.6 $\pm$ 3.0% TCO_2 in 28days Positive control substance: 76.9 $\pm$ 9.5% % in 28 days Final pH: 6.37
Conclusions	The test substance was not readily biodegradable.
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 11/21/2002

3.0 AQUATIC ORGANISMS

3.1 Acute Toxicity to Fish

Robust Summary 10-FISH-1

Test Substance	
CAS #	398141-87-2
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline followed	OECD 203; EPA OPPTS Test Guideline 850.1075
Test Type	Acute Toxicity to Fish
GLP (Y/N)	Y
Year (Study Performed)	2005 - 2006
Species/Strain	Fathead minnow (*Pimephales promelas*) Juveniles were obtained from an inhouse colony.
Fish Size	Average length 2.5 cm and a mean weight of 0.12 g, loading rate of 0.039 g body weight/L (based on 10 negative control fish).
Number of Fish	Definitive Study 20/concentration (10/replicate)
Analytical Monitoring	Yes, for Total Organic Carbon (TOC) at 0, 48 and 96 hours using a total organic carbon analyzer.
Nominal Test Substance Concentration Levels	0, 0.38, 0.75, 1.5, 3.0, 6.0, 12 mg/L
Test Concentration Preparation	Individual test concentrations were prepared for each test level. A measured volume of test material was added to 15 mL of dilution water and sonicated for 2 minutes. This was then rinsed into the test chamber and brought to a volume of 30 L. The 30 L solution was then stirred with a top-down electric mixer for 2-3 minutes.
Exposure Period	96 hours
Exposure Conditions	Static non-renewal test conditions.
Vehicle	None
Statistical Analysis	LC50 and 96% confidence interval determined by binomial probability method with nonlinear interpolation. The no-mortality concentration and NOEC were determined by visual interpretation of mortality and observation data.
Dose Range finding Study	Yes
Test Chambers	38-liter stainless steel aquaria containing 30 L of the test solution
Diluent Water	Fresh aerated well water
Diluent Water Chemistry	Hardness: 138 mg/l as $CaCO_3$ Specific Conductance: 300 µmhos/cm
Diluent Water Chemistry During 96 Hour Exposure Period.	Dissolved Oxygen: 7.5-8.7 mg/L ($\geq$87% saturation). pH: 8.5-8.6
Photoperiod	16 hours of light, 8 hours of dark
Temperature Range	21.0-22.4^{O}C during exposure period
Remarks field for test conditions	All organisms were observed for mortality and the number of individuals exhibiting clinical signs of toxicity or abnormal behavior at 3, 24, 48, 72, and 96 hours after initiation of test material exposure.

Results	All test solutions were clear and colorless at test initiation and termination. Measurements of TOC in the newly prepared test solutions on Day 0 ranged from <1 to 4.1 mg C/L. Measurements of TOC in the 48-hour old solutions on Day 2 ranged from <1 to 4.8 mg C/L. Measurements of TOC in the 96-hour old solutions on Day 4 ranged from <1 to 4.0 mg C/L. TOC content generally increased with increasing test substance concentration and was generally consistent over time. Cumulative mortality data was as follows: Cumulative mortality (%) Concentration (mg/L) Number of Fish 24 Hours 96 Hours 0 20 0 0 0.38 20 0 0 0.75 20 0 0 1.5 20 0 0 3.0 20 0 0 6.0 20 100 100 12 20 100 100 . No signs of toxicity were evident at concentrations up to and including 1.5 mg/L. At 3.0 mg/L 35% of the fish exhibited loss of equilibrium and surfacing at study termination. The No Observed Effect Level, based on mortality and the absence of any sub lethal effects of exposure was 1.5 mg/L. The no mortality level was 3.0 mg/L. The LC50 value at 24, 48, 72 and 96 hours was 4.2 mg/L.
Conclusions	The No Observed Effect Level, based on mortality and the absence of any sub lethal effects of exposure was 1.5 mg/L. The no mortality level was 3.0 mg/L. The LC50 value at 24, 48, 72 and 96 hours was 4.2 mg/L.
Data Quality	Reliable without restriction (Klimisch Code)
References	Palmer & Krueger. (2006). "A 96-hour Static Acute Toxicity Testwith the Fathead Minnow (*Pimephales promelas*)"Wildlife International Project No.: 264A-109
Other	Prepared: 2/27/2005

3.2 Acute Toxicity to Invertebrates (e.g. Daphnia)

Robust Summary 10-Daph-1

Test Substance	
CAS #	398141-87-2
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #202 Daphnia sp. Acute Immobilization Test and Reproduction Test (1984). EPA OPPTS Test Guideline 850.1010
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	2005-2006
Species/Strain	Daphnia magna (Cladoceran) Juveniles were obtained from an in-house colony.
Number of Daphnia	Definitive Study 20/concentration (10/replicate)
Analytical Monitoring	Yes, for Total Organic Carbon (TOC) at 0 and 48 hours using a total organic carbon analyzer.
Exposure Period (unit)	48 hours
Nominal Test Substance Concentration Levels	0, 0.31, 0.63, 1.3, 2.5, 5.0 and 10.0 mg/L
Test Concentration Preparation	A primary stock solution was prepared at a nominal concentration of 10 mg/L, the highest concentration tested, by mixing 30.0 mg of test material into 3 L of dilution water (UV sterilized well water). The stock solution was sonicated for one minute and mixed with a top-down stainless steel mixer. Aliquots of the stock were proportionally diluted with dilution water to prepare 500 mL of test solution at nominal concentrations of 0.31, 0.63, 1.3, 2.5 and 5.0 mg/L. The primary stock solution was mixed continuously during preparation of the test solutions.
Statistical methods	Based on the pattern of mortality/immobility in this study, probit analysis was used to calculate the 24-hour EC50 value and binomial probability was used to calculate the 48-hour EC50value. The no-mortality/immobility concentration and the no-observed-effect concentration were determined by visual interpretation of the mortality, immobility and observation data.
Test chambers	Test chambers were 250-mL glass beakers containing 250 mL of test water. The depth of the test water in a representative chamber was 5.9 cm.
Diluent Water	Fresh aerated well water
Diluent Water Chemistry	Hardness: 136 mg/l as $CaCO_3$ Alkalinity: 182 mg/l as $CaCO_3$ Specific Conductance: 310 µmhos/cm Total Organic Carbon <1 mg C/L
Diluent Water Chemistry	Dissolved Oxygen: 8.3-8.8 mg/L ($\geq$ 92% of saturation) pH: 8.2-8.6

<table>
<tr><td>During 48 Hour Exposure Period.</td><td></td></tr>
<tr><td>Photoperiod</td><td>16 hours of light, 8 hours of dark</td></tr>
<tr><td>Temperature Range</td><td>19.8-20.5^{O}C during exposure period</td></tr>
<tr><td>Remarks</td><td>Observations were made periodically to determine the numbers of dead and immobile organisms. Immobility was defined as a lack of movement by the organism except for minor activity of the appendages. The numbers of individuals exhibiting signs of toxicity or abnormal behavior also were evaluated. Observations were made approximately 4, 24 and 48 hours after test initiation.

TOC in the newly prepared test solutions on Day 0 ranged from <1 to 2.9 mg C/L. Measurements of TOC in the 48-hour old solutions on Day 2 ranged from <1 to 4.1 mg C/L. TOC content generally increased with increasing test substance concentration.

Temperature was maintained at 19.8-20.5^{O}C throughout the test. No treatment related differences were observed in oxygen concentration or pH during the study. The solutions appeared clear and colorless at test initiation and termination.

Cumulative mortality/immobilization data was as follows:

<table>
<tr><th rowspan="2">Concentration (mg/L)</th><th rowspan="2">Number of Daphnia</th><th colspan="3">Cumulative Immobilization/Mortality (%)</th></tr>
<tr><th>4 Hours</th><th>24 Hours</th><th>48 Hours</th></tr>
<tr><td>0</td><td>20/intrerval</td><td></td><td></td><td></td></tr>
<tr><td>0.31</td><td>20/intrerval</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>0.63</td><td>20/intrerval</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>1.3</td><td>20/intrerval</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>2.5</td><td>20/intrerval</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>5.0</td><td>20/intrerval</td><td>0</td><td>35</td><td>60</td></tr>
<tr><td>10</td><td>20/intrerval</td><td>0</td><td>70</td><td>100</td></tr>
</table>

While no mortality/immobility occurred in the 1.3 and 2.5 mg/L treatment groups, 25 and 75% of the daphnids, respectively, appeared lethargic at test termination. Surviving daphnids in the 5.0 mg/L treatment group also appeared lethargic at test termination.

The no-mortality/immobility concentration was 2.5 mg/L and the NOEC was 0.63 mg/L. The 24 and 48-hour EC50s were 7.0 (5.7-9.3) and 4.6 (2.5-10) mg/L.</td></tr>
<tr><td>Conclusions</td><td>The 48-hour EC50 value was 4.6 mg/L, with a 95% confidence interval of 2.5 to 10 mg/L. The no-mortality/immobility concentration was 2.5 mg/L and the NOEC was 0.63 mg/L.</td></tr>
<tr><td>Data Quality</td><td>Reliable without restriction (Klimisch Code).</td></tr>
</table>

References	Palmer & Krueger. (2006) "A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna).*" Wildlife international Project No.: 264A-108.
Other	Updated: 2/28/2006

3.3 Acute Toxicity to Algae

Robust Summary 10-Algae-1

Test Substance	
CAS #	18760-44-6
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test (Water Accommodated Fraction- WAF)
GLP (Y/N)	Y
Year (Study Performed)	2002
Species/Strain	Freshwater algae, *Scenedesmus subspicatus*/CCAP 276/20
Element basis (# of cells/mL)	Approximately 2.45 x 10^6 cells/mL, 5 mL used to inoculate 1 liter of medium for an initial cell density of 10^4 cells/mL.
Exposure period/duration	72 hours
Range find test	Yes
Analytical monitoring	Not performed
Statistical methods	One-way analysis of variance, Bartlett's test and Dunnett's test were used to compare the area under the growth curve data of the treated and control groups.
Remarks field for test conditions (fill as applicable)	Test Species: Cultures obtained from the Culture Collection of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, The Ferry House, Far Sawrey, Ambleside, Cumbria, U.K. Loading Concentrations: 0.313, 0.625, 1.25, 2.5, 5.0 and 10 mg/L loading rate WAF. Test System: The WAF was prepared only at the beginning of the test. A measured weight of test material was added to a measured volume of culture medium (10-L) in a glass vessel and stirred for 24 hours. Stirring accomplished using a magnetic stirrer. Mixing speed was adjusted such that a slight vortex formed. Following the mixing period, the test solutions were allowed to stand for one hour. A small amount of each WAF was removed and examined microscopically for the presence of micro-dispersions or globules of test material. None were observed therefore the WAF was removed from each concentration by mid-depth siphoning. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL. The 250-mL conical flasks were plugged with polyurethane foam bungs. During the test all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 150 cycles per minute under constant light (24 hours/day) for 72 hours. Cell densities were determined using a Coulter Multisizer II Particle Counter at 0, 24, 48 and 72 hours. pH was determined at 0 and 72 hours.

	Light: Continuous illumination approximately 7000 lux. Test temperature: 21.0^{o} C. Culture Media: As specified in the guideline. Method of calculating mean measured concentrations: not applicable Exposure period: 72 hours
Results	EL50(72 hrs)= 3.5 mg/L loading rate WAF [Loading rate that reduced the biomass by 50%]. EL50(0-72 hrs)= 63 mg/L loading rate WAF [Loading rate that reduced specific growth rate by 50%, determined by extrapolation as no concentration resulted in greater than 50% growth inhibition]. There were no statistically significant differences in the area under the growth curve data between the control and 0.313 mg/L WAF test group, however all other loading rates were significantly reduced compared to control. Therefore the No Observed Effect Loading Rate (NOEL) was 0.313 mg/L WAF. The cell concentrations of the control cultures increased by a factor of 69 during the study meeting the guideline requirement of at least a factor of 16 after 72 hours. All test and control cultures were inspected microscopically at 72 hours. No abnormalities were observed in any cultures. Control culture pH increased from 7.4 at 0 hour to 7.9 at 72 hours. This is consistent with the guideline. In the test cultures pH increased over the 72 hour test period following a concentration dependent pattern. Greater increases were observed at lower concentrations. This was attributed to a greater number of viable cells at lower concentrations with greater utilization of carbonates and bicarbonates from respiration.
Conclusions	Both biomass and growth rate were affected by the presence of the test material. EL50 (72 hrs)= 3.5 mg/L loading rate WAF EL50 (0-72 hrs)= 63 mg/L loading rate WAF No Observed Effect Loading Rate (NOEL) = 0.313 mg/L loading rate WAF Control response was satisfactory.
Data Quality	(1) Reliable without restriction
References	Confidential business information.
Other	Updated: 11/19/2002

4. Toxicity

4.1 Acute Toxicity

4.1.1 Acute Oral Toxicity

Robust Summary 10-Acute Oral-1

Test Substance	
CAS #	CAS# 18760-44-6
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline followed	FHSA 16CFR1500.3
Test Type	Acute oral toxicity
GLP (Y/N)	N
Year (Study Performed)	1975
Species/Strain	Rats/Wistar
Sex	Male/female
No. of animals/sex/dose	5
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	0.67, 1.25, 2.5, 5.0 and 10 ml/kg
Dose volume	Not specified
Control group included	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted male and female rats at each treatment level. A control group was not included. The animals were observed for signs of toxicity and mortality for a total of fourteen days. Individual weights were recorded at termination. All animals were euthanized at the conclusion of the observation period. Necropsies were not performed.
Results	Oral LD50 > 10 g/kg (males and females)
Remarks	All animals survived the duration of the study. There were no signs of toxicity observed in any of the animals. The LD50 was > 10 g/kg (males and females).
Conclusions	The test article, when administered as received to male and female Wistar rats, had an acute oral LD50 > 10 g/kg (males and females).
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the fact that this is a summary report. The report contains group summary data but not individual animal data. This is consistent with standard practice at the time that this study was conducted.
References	Unpublished confidential business information
Other	Updated: 11/15/2002

4.1.2 Acute Dermal Toxicity

Robust Summary 10-Acute Dermal-1

Test Substance	
CAS #	CAS# 18760-44-6
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline followed	Similar to OECD Guideline 402
Test Type	Acute dermal toxicity
GLP (Y/N)	Not specified
Year (Study Performed)	1975
Species/Strain	Rabbits/strain not specified
Sex	Male
No. of animals/group	3
Vehicle	None
Route of administration	Dermal
Dose level	2, 4 and 8 g/kg
Dose volume	Not provided
Control group included	No
Remarks field for test conditions	The test material was applied using a syringe under a rubber sleeve that was snugly fastened around the unabraded clipped trunk of the test animal. The animals were immobilized for a 24-hour period immediately following treatment. At the end of the 24-hour period the sleeves were removed and the animals were returned to their cages for a 14-day observation period during which the animals were observed for evidence of toxicity and mortality.
Results	Dermal LD50 was between 4 and 8 g/kg (males)
Remarks	All animals treated at 2 and 4 g/kg survived the duration of the study. The three animals treated at 8 g/kg died on test days 5, 5 and 7. All animals treated at 2 and 4 g/kg exhibited slight weight gain during the study. No significant signs of toxicity were reported.
Conclusions	The test article, when administered dermally as received to male white rabbits had an acute dermal LD50 of between 4 and 8 g/kg.
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the failure to include individual animal clinical data in the report. This is consistent with standard practice at the time that this study was conducted.
References	Unpublished confidential business information
Other	Updated: 11/15/2002

4.2 Repeated Dose Toxicity

Robust Summary 1-Repeated Tox-1

Test Substance	
CAS #	CAS# 18760-44-6
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline followed	OECD 407
Test Type	28-day oral toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	2004
Species	Rat
Strain	Sprague-Dawley Crl:CD IGS BR, Approximately 7 weeks of age at initiation of treatment
Route of administration	Oral gavage
Duration of test	28 days of treatment
Doses/concentration levels	0, 100, 500 and 1000 mg/kg/day
Dose Formulation Analysis	Analysis performed for dosing solution stability, homogeneity and concentration.
Sex	Males and females
Exposure period	28-day treatment duration
Frequency of treatment	Once daily, 7 days/week
Control group and treatment	5 rats/sex/group for each dose group. Control group received daily doses of corn oil at 10.0 ml/kg, and treatment groups received the indicated dose of test material diluted in corn oil at a dose volume of 10.0 ml/kg
Dose Range find Study	Yes
Post exposure observation period	None
Statistical methods	Body weight, body weight change, food consumption, functional observational battery observations, hematology, coagulation and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Tukey-Kramer test, non-parametric Kruskal-Wallis and a Dunn's test, Fisher's Exact test, Chi-Square test, and Levene's test as appropriate.
Remarks field for test conditions	Single oral gavage doses were administered for 28 consecutive days. Clinical observations were made daily, between one-half and two hours following dosing. Detailed clinical observations were performed on day 0, 6, 13, 20 and 27. Viability checks were performed twice daily. An abbreviated functional observation battery (FOB) was performed once prior to dosing initiation and on days 6, 13 and 20. A full FOB assessment (home cage, removal from home cage, open field, manipulative tests and motor activity) was performed on day 27. All FOB assessments were performed blind. Body weights were recorded on test days –1, 6, 13, 20, and 27. Fasted body weights

	were recorded at necropsy. Food consumption was recorded on test days 0, 6, 13, 20, and 27. . Hematology, coagulation and clinical chemistry parameters were evaluated at termination of treatment. Macroscopic examinations were performed on all animals. Select organs were weighed. All tissues were examined microscopically in the control and high dose groups. The kidneys, liver and thyroid were evaluated in all animals.
Results	
Remarks	One mid dose female (500 mg/kg/day) died prior to necropsy following bleeding for clinical pathology. This was considered an accidental death. All of the other control and treated animals survived the duration of the study. Clinical observations that were considered likely to be related to test article administration included salivation prior to dosing, struggling during dosing, and salivation at one-half hour to two hours post dosing. These findings were noted primarily in the animals treated at 1000 mg/kg/day. The 1000 mg/kg/day males had statistically lower levels of aspartate aminotransferase and statistically higher levels of total bilirubin, globulin, and total protein compared to control males. The 500 and 1000 mg/kg/day males had statistically elevated levels of potassium, calcium, and phosphorus compared to the control males. The 500 and 1000 mg/kg/day females had statistically elevated levels of total bilirubin, total protein, and albumin and statistically decreased levels of chloride compared to the control females. All test article-treated female groups had statistically higher levels of calcium relative to controls. The 1000 mg/kg/day females had statistically elevated levels of phosphorus relative to controls. With the exception of the differences in total bilirubin in males and females and the male (1000 mg/kg/day) calcium, the magnitude of the differences observed in test article-treated males and females was 65% or less and the aberrant values were well within the range of historical control values, indicating that these differences were of no toxicological significance, although they may have been test article related. Mean absolute liver weight was statistically greater than control in the 500 and 1000 mg/kg/day males and females. The observed increase in liver weight exhibited a dose-response relationship in both sexes. In the high dose, liver weights were increased 52% in the males and 77% in the females compared to controls. Mean absolute kidney weights were also statistically greater than control in the 500 and 1000 mg/kg/day males and in the 1000 mg/kg/day females. A dose-response relationship was evident in both sexes. The 1000 mg/kg/day males had a mean kidney weight 25%

	greater than controls and the 1000 mg/kg/day females had a mean kidney weight that was 20% greater than controls. The liver and kidney-to-body weight ratios were statistically greater than control in the 500 and 1000 mg/kg/day males, and the liver-to-body weight ratio was statistically greater than control in the 500 and 1000 mg/kg/day females. Minimal to mild eosinophilia of hepatocytes was observed in all test article-treated male groups and in the 500 and 1000 mg/kg/day females. This finding was characterized by the hepatocytes having a more homogeneous, amorphous, eosinophilic cytoplasm and at times appearing to be enlarged relative to those of control animals. The Study Director suggested that the increases in liver weights observed in the 500 and 1000 mg/kg/day males and females and the eosinophilia of hepatocytes noted in all test article-treated male groups and in mid and high dose female groups may be due to hepatic microsomal enzyme induction, as both increased weight and eosinophilia are commonly observed in the livers of animals treated with known cytochrome P450 inducers. Minimal hypertrophy of the thyroid follicular epithelium characterized by a diffuse increase in follicular epithelial cell size was observed in one 500 mg/kg/day male, in all 1000 mg/kg/day males, and in one 1000 mg/kg/day female. An increase in the incidence and/or severity of renal hyaline droplets characterized by increases in tubular epithelial cells with cytoplasmic protein droplets and increases in cytoplasmic protein droplets per tubular epithelial cell was observed in all test article-treated male groups. This finding was not observed in female rats. The renal hyaline droplet changes observed in this study are consistent with male rat-specific nephropathy. Male rat-specific nephrotoxicity is not relevant to human risk assessment, since $\alpha 2_{\mu}$-globulin is only produced in appreciable quantities in male rats. Consequently, the Study Director concluded that the renal findings observed in male rats in this study were likely of no significance. No toxicologically relevant differences in mortality, functional observation battery parameters, body weights, body weight changes, food consumption, hematologic parameters, coagulation parameters or red blood cell morphology parameters were observed in test article-treated males or females in this study. Chemical analysis of dosing solutions confirmed that they were homogeneously prepared and stable at the desired concentrations for up to 3 days at room temperature. Concentration analysis confirmed that the dosing solutions were generally within 15.6% of nominal concentrations.

Conclusions	The target organs for toxicity identified in this study were the liver and thyroid in males and females and the kidney in males. Because of the microscopic histopathologic findings in all test article-treated male groups, a no-observed-effect level (NOEL) could not be identified for males in this study. The 100 mg/kg/day dose was considered to be a no-observed-adverse effect level (NOAEL) in males. The NOEL for females in this study was 100 mg/kg/day.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 6/21/2005; SLI Study No.: 3547.52

4.3 Toxicity to Reproduction

Robust Summary 1-ReproTox-1

Test Substance	
CAS #	CAS# 398141-87-2 / 18760-44-6
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline	OECD Guidelines 421
Test Type	Reproduction/developmental screening study in rats
GLP (Y/N)	Y
Year (Study Performed)	2005-2006
Species	Rat
Strain	Sprague-Dawley CD, 72 days of age at initiation of treatment
Route of administration	Orally by gastric intubation
Duration of test	The males were dosed during study days 0-55 (14 days prior to pairing through 1 day prior to scheduled euthanasia) for a total of 56 doses. The females were dosed from study day 0 through the day prior to euthanasia (14 days prior to pairing through lactation day 3) for a total of 39-53 doses. The female that failed to deliver was dosed through the day prior to euthanasia (post-mating day 25) for a total of 40 doses.
Dose levels	0, 50, 175 and 600 mg/kg/day
Vehicle control	Corn oil
Dose volume	5 mL/kg
Sex	Males and females
Frequency of treatment	Once/day, 7 days/week
Analytical confirmation of concentration.	Homogeneity, stability and dose concentration confirmation
Control and treatment groups	12/sex/group
Post exposure recovery period	None
Mating ratio	One male to one female
Duration of mating period	Up to 14 days with the same male
Statistical methods	Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test article-treated group to the control group by sex. Data obtained from nongravid females were excluded from statistical analyses following the mating period. Where applicable, the litter was used as the experimental unit. Parental mating, fertility, conception and copulation indices were analyzed using the Chi-square test with Yates' correction factor. Mean parental body weights (weekly, gestation and lactation), body weight changes and food consumption, offspring body weights and body weight changes, gestation length, numbers of corpora lutea, implantation sites, number of pups born, live litter size on Post natal day 0, unaccounted-for sites, absolute and relative organ weights and pre-coital intervals were subjected to a parametric one-way analysis of variance to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test was used to compare the test article-treated groups to the control group. Mean litter proportions (percent per litter) of males at birth and postnatal survival were subjected to the Kruskal-Wallis nonparametric ANOVA to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunn's test was used to compare the test article-treated groups to the control group. Histopathological findings in the test article-treated groups

	were compared to the control group using a two-tailed Fisher's Exact test.
Dose range finding study	Dosage levels were selected based on the results of previous studies.
Remarks field for test conditions	Viability and Toxicity: Twice daily Clinical Observations: At lease once daily throughout the study. Body Weights: Individual male body weights were recorded weekly throughout the study and prior to scheduled euthanasia. Individual female body weights were recorded weekly until evidence of copulation was observed. Once evidence of mating was observed, female body weights were recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and on lactation days 1 and 4. Food Consumption: Individual food consumption was recorded weekly until pairing. Food intake was not recorded during the mating period. Once evidence of mating was observed, female food consumption was recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and on lactation days 1 and 4. Following mating, food consumption for all males was measured on a weekly basis until the scheduled euthanasia. Macroscopic Examinations: Performed on all F0 animals. Pup/Litter Examinations: Litters were examined daily for survival and any adverse changes in appearance or behavior. Each pup received a detailed physical examination on postnatal day 1 and 4. Any abnormalities in nursing behavior were recorded. Litter Size: Number of live and dead pups recorded on days 0 and 4 of lactation. Individual Pup Body Weights: Pups were individually weighed on postnatal day 1 and 4. Necropsy: On postnatal day 4, surviving F1 rats were euthanized via an intraperitoneal injection of sodium pentobarbital. Macroscopic Examinations: A macroscopic *post-mortem* examination of the principal thoracic and abdominal organs (with particular attention to the reproductive organs) was performed on all parent animals including any which were sacrificed prematurely. The number of implantation sites and *corpora lutea* was recorded, for all the females, whenever possible. In the females which were apparently non-pregnant, the presence of implantation scars on the uterus was checked using ammonium sulphide staining technique. Pups found dead and pups sacrificed on day 6 post-partum (or shortly hereafter) were carefully examined externally for gross external abnormalities, and a macroscopic examination was performed. Tissues were not retained. Microscopic Examinations: Microscopic examination was performed on the male that was found dead and on all animals in the control and 600 mg/kg/day groups at the scheduled necropsies; gross lesions from all dosage groups were also examined. Because of test article-related findings in the 600 mg/kg/day group males, the thyroid glands were also examined from all males in the 50 and 175 mg/kg/day groups.

Results	Analysis of dosing solutions confirmed that the preparations were homogeneous and stable for their intended period of use and that they were at the appropriate concentrations. One male in the 600 mg/kg/day group was found dead on study day 32 due to complications from fractured nasal bones; the death was not attributed to the test article. All other animals survived to the scheduled necropsy. Salivation and clear material around the mouth were noted in the 175 and 600 mg/kg/day group males and females at the time of and/or 1-2 hours following dose administration. Salivation was observed on at least 1 occasion each in 9 and 4 males and females, respectively, in the 175 mg/kg/day group and in 12 and 11 males and females, respectively, in the 600 mg/kg/day group. Clear material around the mouth was also observed on at least 1 occasion each in 10 and 8 males and females, respectively, in the 175 mg/kg/day group and in all males and females in the 600 mg/kg/day group. The severity of salivation and clear material around the mouth was primarily slight or moderate in both groups. These findings were attributed to the test article, but were not considered adverse because the onset occurred immediately following dose administration; thus these findings were not considered indicative of systemic toxicity. Red material around the mouth (slight or moderate in severity) was also noted 1-2 hours following dose administration in 6 and 10 males and females, respectively, in the 600 mg/kg/day group. Slightly lower mean body weight gains were noted in the 600 mg/kg/day group males during the pre-mating period and when the entire treatment period was evaluated. The reductions in the males in this group were due to transient lower mean body weight gains during study days 7-13 and 21-27. However, the reductions in mean body weight gain were not of sufficient magnitude to result in substantially lower mean body weights in the 600 mg/kg/day group males; therefore, the lower mean body weight gains observed in these males was not considered adverse. No test article-related effects were observed on mean body weights or body weight gains during the pre-mating, gestation or lactation periods in the 50, 175 or 600 mg/kg/day group females. Food consumption in the males and females was not adversely affected by test article administration at any dosage level. Higher mean absolute and relative (to final body weight and to brain weight) thyroid gland weights were noted in the 50, 175 and 600 mg/kg/day group males. Potentially test article-related higher mean absolute and relative (to final body weight and to brain weight) thyroid gland weights were noted in the 175 and 600 mg/kg/day group females. The increases occurred in a dose-related manner and were more pronounced in the males. However, the increases in the females and the 50 mg/kg/day group males were not considered adverse based on the lack of correlating microscopic findings in the 50 mg/kg/day group males and the 600 mg/kg/day group females and no statistical significance. Mean absolute and relative (to final body weight and to brain weight) liver weights were higher in the 175 mg/kg/day group males and the 600 mg/kg/day group males and females. Mean absolute and relative (to final body weight and to brain weight) kidney weights were also higher in the 600 mg/kg/day group males. There were no test article-related macroscopic findings in the males and females at the scheduled necropsy. The mean numbers of former implantation sites and unaccounted-for sites in the test

	article-treated group females were similar to those in the control group. At the microscopic evaluation, follicular cell hypertrophy of the thyroid gland in the 600 mg/kg/day group males correlated with higher mean thyroid gland weights. No test article-related microscopic findings were noted in the thyroid glands of the 50 and 175 mg/kg/day group males. There were no test article-related microscopic findings observed in the 600 mg/kg/day group females. There were no test article-related effects on male and female reproductive performance. The numbers of days between pairing and coitus in the test article-treated groups were unaffected by test article administration. Mean gestation lengths in the 50, 175 and 600 mg/kg/day groups were similar to the control group value; there were no signs of dystocia in this study. The mean numbers of pups born, live litter size on post natal day 0, the percentage of males at birth and postnatal survival in the test article-treated groups were similar to those in the control group. The general physical condition of the F1 pups and mean F1 body weights were not affected by test article administration to the F0 parental animals. There were no macroscopic findings in the F1 pups that were found dead that could be attributed to F0 treatment with the test article.
Conclusions	Based on the results of this study, a dosage level of 600 mg/kg/day (the highest dosage level tested) appeared to be the no-observed-adverse-effect level (NOAEL) for reproductive toxicity. The NOAEL for male systemic toxicity was considered to be 50 mg/kg/day based on increased organ weights in the 175 and 600 mg/kg/day groups and microscopic findings in the 600 mg/kg/day group. The NOAEL for female systemic toxicity was considered to be 175 mg/kg/day based on increased liver weight in the 600 mg/kg/day group. Based on the lack of effects on live litter size, postnatal survival and F1 body weights at any dosage level, the NOAEL for F1 neonatal toxicity was considered to be at least 600 mg/kg/day.
Data Quality	Reliable without restriction (Klimisch Code)
References	Knapp J. (2006.) "A Reproduction/Developmental Toxicity Screening Study of Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide (CAS # CAS# 398141-87-2 / 18760-44-6) in Rats." WIL Research Laboratories LLC Study No. 186044.
Other	Updated: 10/27/06

4.4 Genetic Toxicity

Robust Summary 10-GenTox-1

Test Substance	
CAS #	CAS# 18760-44-6
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline followed	Similar to OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1980
Test System	*Salmonella typhimurium* and *Escherichia Coli*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537; TA1538 *Escherichia Coli* tester strain WP2*uvr*A
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	1, 5, 10, 50, 100, 500, 1000 and 5000 ug/plate
Metabolic Activation	With and without (0.5 mL S9 fraction mix of livers of PCB pretreated Sprague Dawley rats)
Vehicle	Dimethylsulfoxide
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 2-aminoanthracene 0.5 ug/plate TA98 -S9 2-aminofluorene 0.1ug/plate TA100 +S9 2-aminoanthracene 0.5 ug/plate TA100 -S9 2-aminofluorene 0.01ug/plate TA1535 +S9 2-aminoanthracene 2.0 ug/plate TA1535 -S9 N-ethyl-N-nitro-N-nitrosoguanidine 5.0 ug/plate TA1537 +S9 2-aminoanthracene 2.0 ug/plate TA1537 -S9 9-aminoacridine 80.0 ug/plate TA1538 +S9 2-aminoanthracene 0.5 ug/plate TA1538 -S9 2-nitrofluorene 2.0 ug/plate WP2uvrA +S9 2-aminoanthracene 80.0 ug/plate WP2uvrA –S9 2-aminofluorene 0.04 ug/plate
Vehicle Control	Dimethylsulfoxide
Statistical Analysis	Mean revertant colony count was determined for each dose point.
Dose Rangefinding Study	None reported
S9 Optimization Study	None reported
Remarks field for test conditions	This study was conducted prior to the development of OECD Test Guideline 471. The study included the use of tester strain TA1538. OECD 471 does not incorporate this strain. This deviation from the test guideline was not considered a major study deficiency. There were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with eight concentrations of test substance, vehicle controls, and a positive control. Two plates/dose group/strain/treatment set were evaluated. 0.1 mL of test material, positive control or vehicle control were added to each plate along with 0.1 ml of tester strain, 0.5 mL of

	S9 mix (if needed) and 2.0 ml of top agar. This was overlaid onto the surface of minimal bottom agar in a petri dish. A sterility culture was also prepared. Plates were incubated for 48 hours at 37°C. The revertant colonies on the test plates and the control plates were then counted. The test substance was considered positive if the number of revertant colonies (mean value) was more than twice that of the solvent control and exhibited a dose response.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	The test substance failed to exhibit a positive response with or without metabolic activation at any concentration tested. The positive control for each respective test strain exhibited at least a 2-fold increase (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.
Conclusions	Under the conditions of this study, the test material was not mutagenic with or without metabolic activation.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 11/19/2002

Robust Summary 10-GenTox-2

Test Substance	
CAS #	CAS# 18760-44-6 or 398141-87-2
Chemical Name	Thiophene, 3-(decyloxy)tetrahydro-, 1,1-dioxide
Method	
Method/Guideline followed	OECD Guideline 473
Test Type	In Vitro Chromosomal Aberration Assay
GLP (Y/N)	Y
Year (Study Performed)	2006
Test System	Human peripheral blood lymphocytes
Exposure Method	Dilution
Test Substance concentration levels	4 hour treatment, 20 hour harvest without activation: 25, 50, 75, 100, 125, 150, 175 µg/mL 20 hour treatment, 20 hour harvest without activation: 6.25, 25, 50, 65, 75 µg/mL 4 hour treatment, 20 hour harvest with activation (S9): 25, 50, 75, 100, 125, 150, 175 µg/mL
Metabolic Activation	With and without S9 fraction mix of livers of Aroclor 1254-induced rats
Vehicle	DMSO
Vehicle and Positive Control concentration levels by activation status	Mitomycin C - non-activated test system positive control (0.3 or 0.6 µg/mL) Cyclophosphamide - activated test system positive control (20 µg/mL) DMSO – solvent control
Statistical Analysis	Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test. Fisher's exact test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's exact test at any test article dose level, the Cochran-Armitage test was used to measure dose-responsiveness.
Preliminary Toxicity Dose Range Finding Assay	Consisted of an evaluation of test article effect on mitotic index. Evaluation performed at 4 hours with and without activation and following a continuous 20-hour exposure without metabolic activation. Concentrations of test material evaluated ranged from 0.276 to 2760 µg/mL.
Remarks field for test conditions	In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. Mitomycin C (positive control) was tested without activation and Cyclophosphamide (positive control) was tested with activation. Two hours prior to harvest the spindle inhibitor, Colcemid, was added to each culture to obtain a final concentration of 0.1 ug/mL. Slides were prepared using Giemsa stain. Two-slides/treatment group were evaluated. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid type and chromosome-type aberrations. Chromatid type aberrations included chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations included chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were

	not scored as an aberration but instead were considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells (≥ 10 aberrations) also were recorded. Chromatid and isochromatid gaps were not included in the analysis. The percent polyploid and endoreduplicated cells was evaluated per 100 cells. The number and types of aberrations per cell, the percentage of structurally and numerically damaged cells (percent aberrant cells), and the frequency of structural aberrations per cell (mean aberrations per cell) in the total population of cells examined was calculated and reported for each treatment group. Chromatid and isochromatid gaps were not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell. The test article was considered to induce a positive response when the percentages of cells with aberrations were increased in a dose-responsive manner with one or more concentrations being statistically elevated relative to the solvent control group. The test article was concluded to be negative if no statistically significant increase was observed relative to the solvent control.
Results	Under the conditions of this study the test material was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes.
Remarks	The test article was soluble in DMSO and in the treatment medium at all concentrations tested at the beginning of the treatment period. At the conclusion of the treatment period, visible precipitate was observed in treatment medium at dose levels ≥ 276 µg/mL and dose levels ≤ 82.8 µg/mL were soluble in treatment medium. Hemolysis was also observed at dose levels ≥ 276 µg/mL in all treatment groups. The osmolality in treatment medium of the highest concentration tested, 2760 µg/mL, was 406 mmol/kg. The osmolality of the solvent (DMSO) in the treatment medium was 412 mmol/kg. The osmolality of the test article concentrations in treatment medium were acceptable because they did not exceed the osmolality of the solvent by more than 20%. The pH of the highest concentration of test article in treatment medium was approximately 7.0. Substantial toxicity (at least 50% reduction in mitotic index relative to the solvent control) was observed at doses ≥ 276 µg/mL in the non-activated and S9-activated 4-hour exposure groups, and at dose levels ≥ 82.8 µg/mL in the non-activated 20-hour exposure group. In the chromosome aberration assay, the test article was soluble in DMSO and in the treatment medium at all concentrations tested at the beginning and conclusion of the treatment period. Slight hemolysis was observed at 175 µg/mL in the non-activated and the S9-activated 4-hour exposure groups. The osmolality in treatment medium of the highest concentration tested, 175 µg/mL, was 414 mmol/kg. The osmolality of the solvent (DMSO) in the treatment medium was 413 mmol/kg. The pH of the highest concentration of test article in treatment medium was approximately 7.0. At the highest test concentration evaluated microscopically for chromosome aberrations, 100 µg/mL,

	mitotic inhibition was 56%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 25, 50 and 100 µg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level and was within the range for the historical control values. In the S9-activated group at the highest test concentration evaluated microscopically for chromosome aberrations, 100 µg/mL, mitotic inhibition was 52%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 25, 50 and 100 µg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level. At the highest test concentration evaluated microscopically for chromosome aberrations of the non-activated 20-hour exposure group, 50 µg/mL, mitotic inhibition was 51%, relative to the solvent control. The dose levels selected for analysis of chromosome aberrations were 12.5, 25 and 50 µg/mL. The percentage of cells with structural or numerical aberrations in the test article-treated group was not significantly increased above that of the solvent control at any dose level and was within the range for the historical control values. Positive and vehicle control group responses were as expected.
Conclusions	Under the conditions of this study the test material was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes in the presences and absence of a liver metabolizing system at dose levels that induced acceptable levels of toxicity.
Data Quality	Reliable without restriction (Klimisch Code)
References	Gudi & Rao. (2006.) "*In Vitro* Chromosome Aberration Test." BioReliance Study No. AB23NU.341.BTL
Other	Updated: 5/12/06